

Characterization of Broad Spectrum Potato Virus Y Resistance in a *Solanum tuberosum* ssp. *andigena*-Derived Population and Select Breeding Clones Using Molecular Markers, Grafting, and Field Inoculations

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Abstract PVY causes yield and quality loss in potato. The *Ry_{adg}* gene from *Solanum tuberosum* ssp. *andigena* has been shown to provide extreme resistance to PVY; defined as resistance against all strains. However, *Ry_{adg}* gene clones have not been screened against PVY^{N:O}, a newly detected North America strain. Three *Ry_{adg}*-diagnostic molecular markers were tested in tetraploid progeny of a PVY resistant x susceptible cross and in diverse clones/cultivars. Multiple isolates of PVY strains (PVY^{NTN}, PVY^{N:O}, PVY^O) were used for mechanical inoculations of the progeny. In addition, PVY^O, PVY^N, and PVA were used for graft inoculation on a separate clone/cultivar set. Progeny segregated 1:1 for PVY resistance; fitting a gene model simplex for *Ry_{adg}*. Marker positive progeny were resistant to all PVY strains, including PVY^{N:O}. Marker presence was also in agreement with PVY resistance in the clones/cultivars. These findings show that these markers can identify resistance to all known PVY strains in North America.

Resumen El virus Y de la papa (PVY) causa reducción en la calidad y el rendimiento. Se ha demostrado que el gen *Ry_{adg}* de *Solanum tuberosum* ssp. *andigena* provee resistencia extrema contra el PVY; definida como resistencia

contra todas las cepas. Sin embargo, no se ha investigado la resistencia de los clones del gen *Ry_{adg}* contra PVY^{N:O}, una cepa de Norte América recientemente detectada. Tres marcadores moleculares de diagnostico del gen *Ry_{adg}* fueron probados en progenie tetraploide de un cruce de PVY resistente x susceptible y en diversos clones/cultivares. Múltiples cepas aisladas de PVY (PVY^{NTN}, PVY^{N:O}, PVY^O) fueron utilizadas para inoculaciones mecánicas de la progenie y PVY^O, PVY^N y PVA fueron usados para inoculación de injertos en un subconjunto de clones/cultivares. La progenie segregó 1:1 para resistencia contra PVY; encajando en un modelo genético simplex para *Ry_{adg}*. Las progenies positivas a los marcadores fueron resistentes a todas las cepas de PVY, incluyendo PVY^{N:O}. La presencia de marcadores también concordaba con la resistencia contra PVY en clones/cultivares. Estos hallazgos demuestran que estos marcadores pueden identificar la resistencia a todas las cepas de PVY conocidas en Norte América.

Keywords PVY · Marker assisted selection · RT-PCR · PVY resistance

Introduction

Potato virus Y (PVY) is a member of the Potyvirus group and is a monopartite, single strand RNA virus occurring world wide (de Bokx and Huttinga 1981). Since the 1950's in Europe and 1990's in North America, new strains of PVY that can produce potato tuber necrotic ringspot disease (PTNRD) have become widespread (Weidemann 1988; McDonald and Kristjansson 1993; Blanco-Urgoiti et al. 1998; Kerlan et al. 1999; Crosslin et al. 2002; Singh et al. 2003; Crosslin et al. 2005). PVY^O is classified as the

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common strain and in North America is the predominant strain. In Europe, PVY^N strains have become predominant in some areas (Kus 1992; Racman et al. 2001). The N superscript refers to the necrotic reaction on tobacco leaves, which have historically been used to separate isolates into different strain types. The necrotic strains have been further subdivided based on recent molecular, serological, and biological data to include PVY^{NTN}, PVY^{N:O} (in N. America), and PVY^N-Wilga (in Europe). Isolates from these subgroups have been shown to cause tuber necrosis characteristic of PTNRD. The N:O and Wilga (Blanco-Urgoiti et al. 1998) strains have been shown to be recombinants of PVY^O and PVY^N (Chrzanowska 1991; Glais et al. 2002; Singh et al. 2003; Lorenzen et al. 2006). PVY^{NTN} also has O and N sequences that can arise by recombination or mutation (Nie and Singh 2003a; Nie and Singh 2003b). RT-PCR primers have been developed that detect recombination junctions (RJ) in N:O with 1 RJ (Nie and Singh 2002) and in NTN with 3 RJs (Glais et al. 2002).

Sources of resistance to PVY have been reported in over 20 species of *Solanum* (Brown and Corsini 2001; Solomon-Blackburn and Barker 2001b; Simko et al. 2007). PVY resistance genes have been identified and mapped from the following species: *Solanum tuberosum* ssp. *andigena*, *S. tuberosum* ssp. *tuberosum*, *S. chacoense*, and *S. stoloniferum* (Simko et al. 2007). Molecular markers have been developed that are closely linked with these R-genes with applicability for marker-assisted selection (MAS) of resistant individuals within a segregating population (Sorri et al. 1999; Kasai et al. 2000; Flis et al. 2005; Song et al. 2005; Dalla Rizza et al. 2006; Sato et al. 2006). Three markers identified with the resistance gene from *S. tuberosum* ssp. *andigena* are RYSC3, RYSC4, and ADG2. The RYSC markers are sequence-characterized amplified regions (SCAR) (Kasai et al. 2000) and the ADG2 marker is a cleaved-amplified polymorphic sequence (CAPS) (Sorri et al. 1999). The resistance from *S. tuberosum* ssp. *andigena*, conferred by the *Ry_{adg}* gene mapped to chromosome XI (Hamalainen et al. 1998), has been classified as extreme resistance (ER) which is defined as resistance to all strains of PVY (Munoz et al. 1975; Gebhardt and Valkonen 2001). Since the emergence of these PVY strains and the opportunity to use MAS in breeding programs, it has become important to validate the broad spectrum resistance afforded by *Ry_{adg}*. Singh et al. (2003) has shown that while PVY^{N:O} strains can be detected by current seed potato certification systems utilizing Enzyme Linked Immunosorbent Assay (ELISA) the PVY^{N:O} strains cannot be distinguished from PVY^O with the antisera used for ELISA, allowing the misclassification of PVY^{N:O} as PVY^O in the seed certification system. Researchers who have assayed resistance in clones with the *Ry_{adg}* gene have used PVY^O, PVY^N, and PVY^{NTN}, but not PVY^{N:O} strains or the

analogous PVY^N-Wilga strain (Table 1.) (Munoz et al. 1975; Valkonen 1994; Le Romancer and Nedellec 1997; Hamalainen et al. 1998; Sorri et al. 1999; Kasai et al. 2000; Vidal et al. 2002; Dalla Rizza et al. 2006; Gebhardt et al. 2006). Characterization of a segregating population carrying the *Ry_{adg}* resistance gene has not been done with multiple PVY strains and isolates that include PVY^{N:O}. Effective use of SCAR and CAPS markers for *Ry_{adg}* would aid in identifying resistant individuals early in the breeding process thereby facilitating the development of potato cultivars with extreme resistance to PVY.

The objectives of this work were to; 1) evaluate *S. tuberosum* ssp. *andigena*-derived resistance to multiple PVY strains endemic to North America, and 2) test the effectiveness of three *S. tuberosum* ssp. *andigena* based markers in identifying PVY resistance response among germplasm, advanced breeding clones, and cultivars in the USDA-ARS Aberdeen, Idaho potato breeding program.

Materials and Methods

Plant Material

Plant material used in this study included fifty progeny from family A00664, derived from the intercrossing of PVY resistant R247-1, a tetraploid breeding clone with the *Ry_{adg}* gene, and GemStar Russet, a PVY susceptible cultivar. R247-1 is a clone derived from the cross of breeding clone N537-13 with bulked pollen from *S. tuberosum* ssp. *andigena* (W. De Jong, Cornell University, pers. communication). The fifty progeny of family A00664 were randomly selected in the first field generation and subsequently maintained via greenhouse production of tubers. Before inoculations, plants were ELISA tested for PVY. Resistance to PVY was determined by ELISA and graft inoculation. Additionally, 53 individuals (Table 5) from the USDA-ARS potato breeding program at Aberdeen, Idaho were tested for the *Ry_{adg}* markers to determine if there is agreement between the presence of the markers and known PVY resistance. These plants also were initially field grown and subsequently propagated in the greenhouse. The plants from greenhouse-grown tubers were ELISA tested for PVY before inoculations. These plants included individuals with *S. acaule*, *S. tuberosum* ssp. *andigena*, *S. demissum*, *S. hougasii*, *S. microdontum*, *S. phureja*, *S. stoloniferum*, and *S. vernii* background and PVY susceptible cultivars as controls. Response to infection by PVY in these individuals was based on: 1) field screening trials as described by Corsini et al. (1994), 2) from graft-inoculation studies in the greenhouse, and 3) from the available literature. Two *S. pinnatisectum* clones, PI 275233 and PI253214 (NRSP-6, U.S. Potato Genebank,

Table 1 PVY strains used in studies of PVY resistance in clones with *Ry_{adg}* gene mediated resistance

Authors	PVY strain	Inoculation method			ELISA tested		
		graft	rub	aphid	Marker(s)	Other	
Munoz et al. 1975	PVY ^O	X	X	X			
Valkonen 1994	PVY ^O		X				
Le Romancer & Nedellec 1997	PVY ^{NTN}		X		X		
Hamalainen et al. 1998	PVY ^O	X	X		X	ADG1	ADG2
	PVY ^N						
Sorri et al. 1999	PVY ^O	X	X		X	ADG2	
Kasai et al. 2000	PVY*					RYSC3	RYSC4 previous reports
Vidal et al. 2002	PVY ^O		X		X	ADG2	
Dalla Rizza et al. 2006	PVY*				X	RYSC3	M45 previous reports and field obs.
Gebhardt et al. 2006	PVY ^N	X			X	RYSC3	

*strain not stated

Sturgeon Bay, WI, USA), were also screened with the markers to assess their amplification in a previously uncharacterized potato species.

Evaluation of Resistance

Manual Inoculations of Family A00664

Three to five plants of each clone of family A00664 were rub inoculated to assess virus resistance to multiple strains. Virus isolates used in this study are detailed in Table 2 and include 2 PVY^O isolates, 3 PVY^{N:O} isolates, and 3 PVY^{NTN} isolates. Plants were 7.6–15.2 cm tall when inoculated. Infected tobacco leaf tissue (Samsun NN) was ground at a ratio of 1 g leaf tissue to 3 ml chilled phosphate buffer (pH 7.5; 0.1 M K₂HPO₄, 0.025 M KH₂PO₄) and filtered through cheesecloth. Three to four leaves from each plant were sprinkled with 320 grit

carborundum and rub inoculated using a small square of sap saturated cheesecloth. Spray inoculation consisted of using 1 g of leaf tissue per 3 ml of phosphate buffer. This infected sap was put in a 207 ml stainless steel automotive “touch-up” paint sprayer and leaves were sprayed at a pressure of 40 psi until all leaves were wet. Leaves were rinsed with water 5 minutes after inoculation. Plants were ELISA tested (Clark and Adams 1977) with monoclonal antibodies for PVY^O and PVY^N obtained from SASA (Edinburgh, Scotland). Plants were tested for the presence of virus two and four weeks following inoculation. Plates included negative and positive controls and an absorbance (A₄₀₅) value greater than 0.1 was regarded as positive for PVY.

Graft Challenge of Select Breeding Program Clones and Cultivars

PVY^O, PVY^N, and PVA isolates were obtained from field-infected tubers in eastern Idaho by Dennis Corsini, USDA-ARS, Aberdeen, Idaho. The PVA isolate was collected from field infected Russet Burbank and was used because resistance to PVA has been shown to be tightly linked to the *Ry_{adg}* gene (6.8 cM distal to the gene) with concurrent expression of resistance to both PVY and PVA having been documented (Hamalainen et al. 1998). The PVY^N isolate (designated RR-1) from field infected Ranger Russet was verified to strain identity by Crosslin et al. (2005). To perform the graft, a 10 cm tall stem was decapitated 4 cm below the growing point. A vertical slit was made in the stem at the decapitation point. Virus infected scions of cv. Russet Burbank which had been trimmed into a wedge shape were inserted into the slit and the union was bound with Parafilm®. Shoots that grew basal to the graft stem were assayed 30 days later. The sampled tissue was tested

Table 2 Biological and serological assay of PVY isolates used to characterize *S. tuberosum* ssp. *andigena*-derived resistance

PVY strain	Isolate	Tobacco reaction	Serotype	Tuber necrosis in Yukon Gold
NTN	FL40D	Necrotic	N	Yes ¹
NTN	RR1	Necrotic	N	Yes ¹
NTN	ID10	Necrotic	N ²	Yes ³
N:O	AL1	Necrotic	O	Yes ³
N:O	180A	Necrotic	O	na
N:O	ME193	Necrotic	N ²	Yes ³
O	WHA99	na	O	na
O	Oz	no necrosis	O	No ¹

¹Baldauf et al. (2006); ²S.M. Gray unpublished; ³J.L. Whitworth unpublished

by ELISA using a two step method (Kaniewski and Thomas 1988). Leaf tissue was ground in phosphate buffer (200 mg/2 ml). PVY antiserum, a polyclonal derived from rabbit, was used at 1/2000 dilution in trapping antibody and conjugate. The reaction was allowed to develop from 30 to 60 min. Five replicate grafts were tested. Materials with all absorbencies (A_{405}) under 0.1 were considered resistant, i.e. extremely resistant (Valkonen et al. 1996), while those above this value were scored as systemically infected. Necrosis and stem death accompanying grafting was interpreted as a hypersensitive reaction.

Field Evaluations for PVY Resistance in Breeding Program Clones and Cultivars

Field evaluations for resistance were initiated by mechanical inoculations with PVY-infected plant sap and aphid inoculations from PVY-infected source plants. Five hill plots with three replications were planted in a randomized complete block design at Kimberly, Idaho. In 2005 and earlier, when plants were 15–20 cm tall, they were rub-inoculated by hand using infected PVY potato leaves. For 2006, plants were inoculated with PVY-infected potato sap in a phosphate buffer delivered through a stainless steel pressurized spray gun with a 1 liter reservoir. The spray gun is powered by an air compressor with pressure adjusted to a minimum of $2.81 \text{ kg}\cdot\text{cm}^{-2}$ (2.76 bar). For the sap-in-buffer, leaf tissue was weighed and blended at a ratio of 1.5 grams tissue in 5.0 ml chilled phosphate buffer (pH 7.5; 0.1 M K_2HPO_4 , 0.025 M KH_2PO_4). Blended material was strained through cheese cloth into a graduated beaker and placed on ice until use. Carborundum (320 grit) was added to the sap at 5 cm^3 per liter and kept in suspension by shaking the container between inoculations. Plants were sprayed with infected sap until the leaves were wet. To facilitate aphid inoculation, spreader rows of Russet Burbank interspersed with PVY-infected seed pieces were planted every third row in the plots and greenhouse-reared green peach aphids (*Myzus persicae*) were placed in the plots approximately five-six weeks post-planting. Aphid-friendly novaluron (Rimon®) and spinosad (Success®) insecticides were used in the plots to control Colorado potato beetles (*Leptinotarsa decemlineata*); no aphid control measures were used. This allowed assessment of PVY resistance under heavy virus pressure provided by infection with mechanical and aphid transmission. At harvest, 10 tubers were saved from each plot and stored. After natural dormancy break, the bud end of each tuber was excised using a tool to cut an approximately 11.4 g spherical seed piece. PVY has been shown to be more concentrated in the bud end of tubers after dormancy break (Gugerli and Gehriger 1980). These seed pieces were put in 10 cm pots

in the greenhouse and grown to approximately 15 cm in size. Leaf tissue from each plant was then ELISA tested for PVY using methods previously described for manual inoculations

Molecular Marker Work

DNA Extraction

DNA extraction was done according to the method described by Bernatzky and Tanksley (1986) except 0.02 M Na-Bisulfite was used instead of B-mercaptoethanol and chloroform/isoamyl alcohol instead of chloroform/octanol and the purified DNA was treated with RNase A instead of CsCL/ethidium bromide centrifugation.

Polymerase Chain Reaction

PCR amplification of markers linked to *Ry_{adg}* was carried out using a PTC-200 thermocycler (Biorad) with primers developed by Kasai et al. (2000) and Sorri et al. (1999). Kasai's SCAR markers identified as RYSC3 and RYSC4 and Sorri's CAPS marker, ADG2/*Bbv*I are tightly linked to and show a high degree of accuracy for detection of the *Ry_{adg}* gene. Each reaction contained 1X REDtaq DNA buffer (Sigma), 0.1 mM dNTP mix, 0.25μM each Primer, 0.05U/μl REDtaq DNA Polymerase, 50 ng template DNA, and ultra-pure H₂O for final reaction volume of 50μl. The PCR program for the SCAR markers consisted of an initial denaturation step at 93°C for 9 min., followed by 35 cycles of denaturation at 94°C for 45 s, primer annealing at 60°C for 45 s, and extension at 72°C for 60 s, followed by a final extension at 72°C for 5 min. PCR products were analyzed by electrophoresis in a 1% agarose gel in 1X TAE. Presence of a 321 base pair (bp) band for RYSC3, and presence of a 145 bp band for RYSC4 was associated with PVY resistance from *Ry_{adg}*. Absence of the amplified products was associated with susceptibility to PVY (Kasai et al. 2000). The PCR program for the CAPS marker consisted of an initial denaturation step at 93°C for 2 min., followed by 40 cycles of denaturation at 93°C for 45 s, primer annealing at 45°C for 45 s, and extension at 72°C for 60 s, followed by a final extension at 72°C for 5 min. The PCR products of ADG2 were digested in a reaction volume of 20μl with 0.05 U/μl *Bbv*I enzyme, 10 X enzyme buffer (NEB2). Samples were digested at 65°C for 3 hours and products were then visualized on a 1% agarose gel in 1X TAE. Presence of an undigested product of 355 bp was associated with the PVY resistance gene *Ry_{adg}*. Presence of one digested product of 270 bp was associated with PVY susceptibility (Sorri et al. 1999).

Results

Molecular Characterization of Family A00664

The presence of alleles associated with PVY resistance at all three markers associated with *Ry_{adg}* were present in 21 of 50 clones of family A00664, with the remaining 29 clones lacking them—indicative of tight linkage among the three markers (Fig. 1). Thirty two of the fifty clones were challenged with multiple isolates of PVY^O, PVY^{N:O}, and PVY^{NTN}. For each virus isolate evaluated, results show that the A00664 *S. tuberosum* ssp. *andigena*-derived population has a 1R:1 S ratio confirming a simplex ratio characteristic of resistance controlled by a single dominant gene (Table 3). SCAR markers showed different intensities in the gels, but the relative band intensity was similar for each lane/clone with both markers, regardless of the gels. With the CAPS marker, more resolution was obtained between bands when the PCR product was precipitated and re-suspended as recommended by Sorri et al. (1999).

The results from ELISA tests of the breeding population for PVY show that presence of all three markers is in agreement with a resistance response (Table 4). None of the clones with all three markers present tested positive for the presence of PVY with ELISA. In the clones with markers absent, ELISA tests for each PVY strain were positive with at least one positive test per virus strain, confirming susceptibility when *Ry_{adg}* genes were absent. One clone was an exception to this; it was negative for PVY^{NTN}

isolates FL40D, RR1, and was not tested for ID-10 due to plant death before assay. Instances of clones testing negative for PVY, although lacking all three bands (Table 4) are thought to be examples of “escapes”—i.e., susceptible individuals in which the inoculation was unsuccessful in eliciting an infectious response.

Breeding Program Clones and Cultivars

Fourteen clones and cultivars from the Aberdeen Breeding Program had both SCAR and the CAPS markers present. Eleven of these clones were PVY resistant in field screening and/or greenhouse grafting trials and had *S. tuberosum* ssp. *andigena* in their pedigrees (Table 5); two of the remaining clones (Costanera, OR000041-4VR) had not been tested for their reported response to PVY infection and a third clone (A85530-10) showed resistance to PVY in the field, but in grafting tests was susceptible to PVY^O and resistant to PVY^N. No bands were present for clones identified as susceptible.

In testing the breeding clones, there are nine cases (Table 6) where clone PI 343201 is in the parentage. The background of PI 343201 includes *S. tuberosum* ssp. *andigena* and *S. stoloniferum*. All *Ry_{adg}* markers are absent in seven and present in two (A88597-7 and AO95496-4) of these nine PVY resistant clones. The seven clones lacking *Ry_{adg}* markers would indicate that their PVY resistance is likely conferred by *Ry_{sto}* and/or other unidentified resistance genes. There are other clones/cultivars with similar

Fig. 1 PCR products from fifty individuals of family A00664 (R241-1 x GemStar Russet) in three different agarose gels of *Ry_{adg}*-based SCAR markers RYSC3 (a) and RYSC4 (b), and CAPS marker ADG2/*Bbv*I (c) (Lanes 1–50). Arrowheads show position of bands associated with PVY resistance derived from *Ry_{adg}*

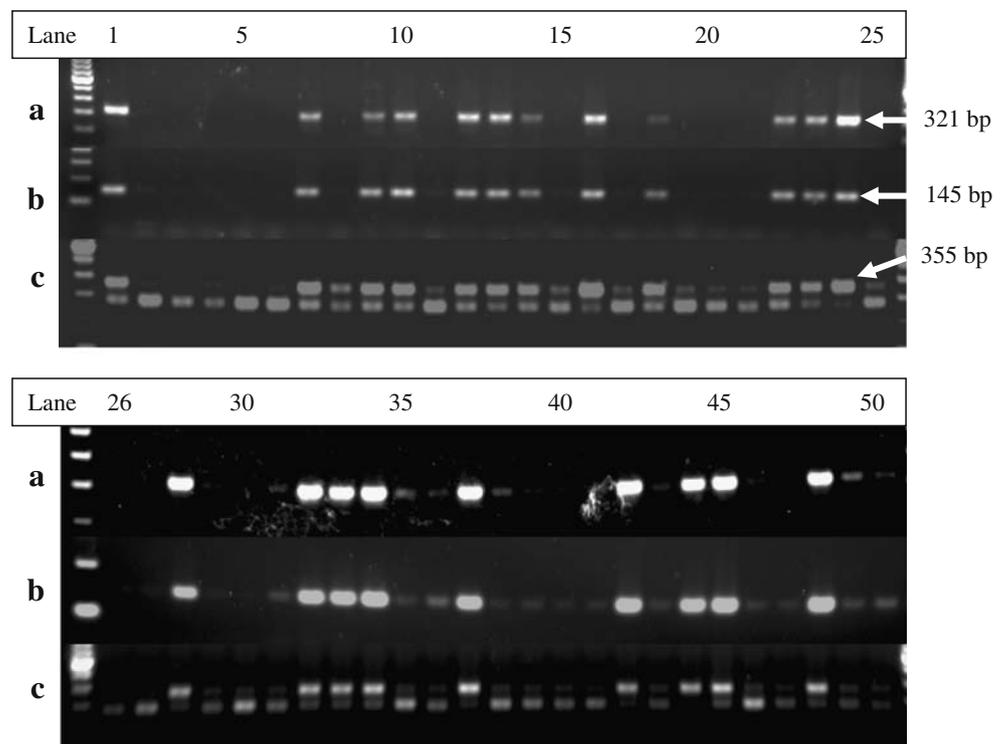


Table 3 Evaluation of PVY resistant model for *S. tuberosum* ssp. *andigena* population (Ho=1R:1S). Percent of resistant progeny and associated chi-square values from a population of cross RY247-1 x GemStar Russet (resistant x susceptible). Results based on ELISA tests for PVY

PVY strain	Isolate	Total number Res	Sus	% Res progeny	χ^2	p value	Ho=1R:1S p<0.05
NTN	FL40D	16	15	51.6	0.063	0.803	Fail to reject
NTN	RR1	16	13	55.2	0.563	0.453	Fail to reject
NTN	ID10	13	12	52.0	0.077	0.782	Fail to reject
N:O	AL1	16	15	51.6	0.063	0.820	Fail to reject
N:O	180A	16	13	55.2	0.563	0.453	Fail to reject
N:O	ME193	14	11	56.0	0.643	0.423	Fail to reject
O	WHA99	16	16	50.0	0.000	1.000	Fail to reject
O	Oz	12	10	54.5	0.520	0.471	Fail to reject

results in Table 5, but these seven clones serve to illustrate the usefulness of *Ry_{adg}* markers for determining whether *Ry_{adg}* is actually contributing to observed PVY resistance in breeding material—useful information for the potato breeder for the pyramiding of divergent sources of PVY resistance.

S. tuberosum ssp. *andigena* is reported to have a resistance gene, *Ra_{adg}*, for PVA resistance closely linked to the *Ry_{adg}* gene for PVY resistance. Results from PVA graft-inoculations also show that of fourteen PVA grafted clones, seven clones had all *Ry_{adg}* markers present, but only four were PVA resistant (Table 5). The remaining seven clones had no markers, but six were PVA resistant and one was PVA susceptible.

The *S. pinnatisectum* clones screened with the markers were both negative for SCAR markers and positive for the CAPS marker.

Discussion

The ability to check for the presence of *Ry_{adg}* before hybridizing parents or before the field screening of progeny can increase selection efficiency for PVY resistance. This data shows a clear agreement between the markers of a *S. tuberosum* ssp. *andigena* derived population from a cross between a PVY resistant clone (R247-1, *andigena* background) and a PVY susceptible clone (GemStar Russet, non-*andigena*). When all three tested markers were present in a clone, PVY resistance (against all three PVY strains), indicated by a negative ELISA test was also present.

Susceptible clones were susceptible (ELISA positive) to all three PVY strains with one exception. One susceptible clone (out of the total sixteen) was susceptible for PVY^O and PVY^{N:O}, but resistant (ELISA negative) for two of three PVY^{NTN} isolates and was not tested for the third isolate due to plant death before testing. The breeding population results indicate that *Ry_{adg}*-mediated resistance confers PVY resistance against all strains (i.e. extreme resistance). This information confirms previous literature, cited in Table 1, showing resistance against different PVY strains and expands the knowledge by including multiple isolates of previously tested strains and PVY^{N:O}, a strain not previously screened against *Ry_{adg}*.

Knowledge of parental background of a clone is important for planning crosses, but the use of markers that can detect when resistant gene(s) are actually present is also important. An example is the identification in this study of PVY resistant breeding material derived from *S. tuberosum* ssp. *andigena* lacking *Ry_{adg}*-associated markers—indicative that observed resistance response in these clones is conferred by PVY resistance genes other than *Ry_{adg}*. Reports of similar disparities between pedigree and actual presence of PVY resistance genes from the presumed source have been reported (Sorri et al. 1999; Kasai et al. 2000).

In the present study, three clones (AWN86514-2, Liu, Serrana), were PVY resistant in field screening but PVY susceptible in greenhouse grafting trials and had no *Ry_{adg}* markers present. These clones were PVY^O graft susceptible, but were not tested against PVY^N. Serrana is listed as by Kasai et al. (2000) as having a PVY hypersensitive

Table 4 Correlation between *Ry_{adg}* markers RYSC3, RYSC4, ADG2/*Bbv*I and response to infection by PVY isolates using ELISA

Clones with:	PVY ^O		PVY ^{N:O}			PVY ^{NTN}		
	WHA	Oz	180A	AL1	ME193	FL40D	RR1	ID10
Markers all present	0/16 ¹	0/12	0/16	0/16	0/14	0/16	0/16	0/13
Markers all absent	16/16	10/13	13/16	15/16	11/14	15/16	13/16	12/13

¹ number of positive ELISA tests/number of clones tested

Table 5 Marker presence/absence in select clones/cultivars from the USDA-ARS Aberdeen breeding program with associated pedigree background and response to PVY infection

Cultivars/clones	Parents		Putative Sources of PVY Resistance ¹	RY SC3	RY SC4	ADG2/ <i>BbvI</i>	PVY Res.	graft results			0% PVY in yrs. ²
	Female	Male						PVY			
								O	N	PVA	
R241-16	N537-13 x	bulk adg	<i>A</i>	+	+	+	R ³	I ³	I	S	11
R247-1	N537-13 x	bulk adg	<i>A</i>	+	+	+	R	I			10
GemStar Russet	Gem Russet	A8341-5	—	—	—	—	S	S			0
A00646-4	A93570-13	A95154-1	<i>A,a,d,s</i>	—	—	—	R				2
A00677-5	PA89A03-4	A92158-3	<i>s</i>	—	—	—	R				1
A00681-4	PA92A08-17	A7961-1	<i>s</i>	—	—	—	R				2
A02768-1	Bannock R.	A93757-4	<i>A</i>	+	+	+	R				1
A85530-10	R247-1	ND860-2	<i>A</i>	+	+	+	R	S	I	S	3
A85536-1	PI343201	Ranger R.	<i>A,a,d,s</i>	—	—	—	R	S			6
A88597-7	A82580-1	R241-16	<i>A,a,d,s</i>	+	+	+	R	I	I	I	5
A88616-4	PI343201	R241-16	<i>A,a,d,s</i>	—	—	—	R				8
A88617-6	PI343201	R247-1	<i>A,a,d,s</i>	—	—	—	R	I	I	S	10
A88625-10	R247-1	A80559-2	<i>A</i>	+	+	+	R	I	I	H	10
A93575-4	A86102-6	A85519-6VR	<i>A</i>	+	+	+	R	I	I	I	2
A95506-1	A88616-4	Ranger R.	<i>A,a,d,s</i>	—	—	—	R	I	I	I	1
A96510-4Y	PA92A17-6	A91194-4	<i>s</i>	—	—	—	R				3
A96510-5	PA92A17-6	A91194-4	<i>s</i>	—	—	—	R				3
A96949-4	AWN 85542-9	Ranger R.	<i>A,a,d,s</i>	—	—	—	R				2
Albatros	1.293112-77	1.3014102-81	<i>A,d,s</i>	—	—	—	R				2
AO95496-4	A88597-7	A85519-6	<i>A</i>	+	+	+	R	I		S	2
AO95506-3	A88616-4	Ranger R.	<i>A,a,d,s</i>	—	—	—	R	I		I	nt
AO95518-1	A8961-14	A88620-6	<i>A,a,d,s</i>	+	+	+	R	I			1
AO95518-2	A8961-14	A88620-6	<i>A,a,d,s</i>	+	+	+	R	I			nt
AO96922-1	Summit R.	A88625-10	<i>A</i>	+	+	+	R	I		H	nt
AWN86514-2	KSA195-96	Ranger R.	<i>A,a,d,s,m,p</i>	—	—	—	R	S			1
B0767-2	B0300-8	Lemhi R.	<i>unknown</i>	—	—	—	R				nt
CO00256-4VR	AO95506-3	CO92027-2	<i>A,a,d,s</i>	—	—	—	R				nt
Costanera	LT-1	PVY+PVX bulk	<i>A</i> ⁸	+	+	+	R				nt
Fasan	6.9152 023-77	1.299 103-77	<i>A,h,s</i> ⁹	—	—	—	R				2
Ida Rose	Sangre	TXA218-7	—	—	—	—	S ⁵				nt
Ivory Crisp	ND292-1	TND22-2	—	—	—	—	S				0
Liu	II70/4154	I65.751/132n	<i>d</i> ⁷	—	—	—	R	S			3
OR000041-4VR	Ranger R.	AO95528-3	<i>A, d, s</i>	+	+	+	R				nt
PA92A08-17	D305.1	A8469-5	<i>s</i>	—	—	—	R	I			4
PA95A14-43	A8259-5	A86102-6	<i>A</i>	—	—	—	S	S			nt
PA98V1-1	PA92A18-17	Ranger R.	<i>s</i>	—	—	—	R	I	I	I	nt
PA98V1-2	PA92A18-17	Ranger R.	<i>s</i>	—	—	—	R	I	I	I	nt
PA98V2-1	PA92A18-17	bulk	<i>s</i>	—	—	—	R	I	I	I	nt
PA98V6-1	PA92 A18-17	A84420-5	<i>s</i>	—	—	—	R	I	I	I	nt
PALB03035-3	POR00LB6-2	GemStar R.	<i>s</i>	—	—	—	R	I			1
PALB03035-7	POR00LB6-2	GemStar R.	<i>s</i>	—	—	—	R	I			1
Ranger Russet	Butte	AA695-3	—	—	—	—	S				0
Reiche	Mex-32	XY.9	<i>A</i> ⁸	+	+	+	R				1
Resurs	69.5403.259	Smena	<i>d</i> ⁷	—	—	—	R				1
Russet Burbank*	Burbank		—	—	—	—	S	S			0

Table 5 (continued)

Cultivars/clones	Parents		Putative Sources of PVY Resistance ¹	RY SC3	RY SC4	ADG2/ <i>Bbv1</i>	PVY Res.	graft results			0% PVY in yrs. ²
	Female	Male						PVY			
								O	N	PVA	
Sante	y66-13-636	AM66-42	<i>A,d,s,v</i> ¹⁰	–	–	–	R				2
Serrana	B2.63	MPI59703/21	<i>d</i> ⁹	–	–	–	R	S#			1
Sheriff	36393	36841	<i>A,d,p</i> ^{4,9}	–	–	–	R				nt
Stirling	8318(6)	8204(4)	<i>d,p</i> ⁴	–	–	–	R				0
Stobrawa	MPI55.957/54	Ora (Mira)	<i>d</i> ⁹	–	–	–	R				2
Umatilla	Butte	A77268-4	—	–	–	–	S				0
Wallowa Russet	A82758-3	Ranger R.	<i>p</i>	–	–	–	MR ⁶				nt
WHA4052-1	R. Norkotah	96-10242	<i>A,p</i>	–	–	–	S				0
Species											
PI275233	collection		<i>pin</i>	–	–	+	U				nt
PI253214	collection		<i>pin</i>	–	–	+	H ¹¹				nt

¹ A-*S. t. andigena*; a-*S. acaule*; d-*S. demissum*; m-*S. Microdontum* (syn. *S. simplicissifolium*); p-*S. phureja*; pin-*S. pinnatisectum*; s-*S. stoloniferum*; v-*S. vernei*; “—” = resistance source unknown

² number of years with 0% PVY in ELISA test of daughter tubers grown-out following field inoculations with PVY^O

³ H=hypersensitive response; R=resistant; S=susceptible; I=Immune; U=unknown

⁴ J Bradshaw, pers. com. and European Cultivated Potato Database

⁵ Love et al. (2002)

⁶ Hane et al. (2003)

⁷ background from van Berloo et al. (2007)

⁸ background from M. Bonierbale and M. Ghislain, pers. com.

⁹ putative background from Hungarian breeding program, H. Junghans (pers. com.)

¹⁰ background from European Cultivated Potato Database

¹¹ USDA-ARS (2008)

*Sport of Burbank; Burbank from Early Rose seedling (Hils and Pieterse 2005)

#Kasai et al (2000), lists as hypersensitive

reaction. PVY hypersensitivity has been characterized as resistance to some but not all PVY strains. In addition, hypersensitivity is expressed as necrotic lesions in inoculated leaves resulting in localized infection (Valkonen et al. 1996). In the field screening, harvested daughter tubers were re-grown, ELISA tested and found negative. In the case of Serrana, it is possible that localized virus was present in the foliage, but it was not translocated to the tubers. In the greenhouse grafting, virus was detected in the shoot tissue basal to the graft, but tubers were not collected. Whichever type of resistance is present, it is from sources other than *Ry_{adg}*.

One clone (A85530-10) out of the fourteen with markers present was field-resistant to PVY and graft-resistant to PVY^N, was graft-susceptible to PVY^O; fitting the definition of hypersensitive resistance; resistant to some but not all virus strains.

Six additional clones with reported resistance were not assayed in the field or greenhouse. Of these six clones,

three had all markers present and would most likely prove PVY resistant once assays are conducted (AO96922-1, Costanera, OR000041-4VR; Table 5). While two of the other three clones (CO00256-4VR, Sheriff; Table 5) have *S. tuberosum* ssp. *andigena* background, absence of markers would indicate that *Ry_{adg}* is not the source of resistance (the third clone, Wallowa Russet, has *S. phureja* background). Other markers that can detect presence of resistance genes, such as *Ry_{sto}*, are valuable and necessary to confirm transmission of those genes to the progeny (Valkonen et al. 2008). Use of the RYSC3 *S. tuberosum* ssp. *andigena* marker and a *S. stoloniferum* (M45) marker in a breeding program has recently been described by Dalla Rizza et al. (2006). Their results show agreement with the RYSC3 and M45 markers in 46 of 47 clones tested. However, Valkonen et al. (2008) suggests that the M45 marker was not specific for *Ry_{sto}*, but for *Ry_{adg}*. The M45 marker was developed in mapping the *Ry_{sto}* gene on chromosome XI (Brigneti et al. 1997). Later Flis et al. (2005) mapped gene *Ry-f_{sto}* to

Table 6 Presence/absence of *Ry_{adg}* markers and response to infection by PVY in clones with common *S. tuberosum* ssp. *andigena* parentage

Cultivars	Pedigree			Background ¹	RY SC3	RY SC4	ADG2 / <i>BbvI</i>	PVY		0% PVY in yrs. ²
	1 st gen.	2 nd gen.	3 rd gen.					O	N	
R241-16				<i>A</i>	+	+	+	I	I	11
R247-1				<i>A</i>	+	+	+			10
A00646-4			PI343201	<i>A,a,d,s</i>	–	–	–			2
A85536-1	PI343201			<i>A,a,d,s</i>	–	–	–	nt	nt	6
A88597-7	R241-16	PI343201		<i>A,a,d,s</i>	+	+	+	I	I	5
A88617-6	PI343201 x R247-1	PI343201		<i>A,a,d,s</i>	–	–	–	I	I	10
A88616-4	PI343201 X R241-16			<i>A,a,d,s</i>	–	–	–			8
A95506-1		PI343201 x R241-16		<i>A,a,d,s</i>	–	–	–	I	I	1
A96949-4		PI343201		<i>A,a,d,s</i>	–	–	–			2
AO95496-4		R241-16	PI343201	<i>A,a,d,s</i>	+	+	+	I	nt	2
AO95506-3		PI343201 X R241-16		<i>A,a,d,s</i>	–	–	–	I	nt	nt

¹ A-*S. t. andigena*, a-*S. acaule*, d-*S. demissum*, p-*S. phureja*, s-*S. stoloniferum*

² number of years with 0% PVY in ELISA test of daughter tubers grown-out following field inoculations with PVY^O

chromosome XII and showed that it was in agreement with Polish and German cultivars that expressed ER to PVY. Therefore, before relying on a specific set of markers in a breeding program, it would be important to test the markers against cultivars with known resistant species background and document resistance responses to the various PVY strains.

The breeding population (A00664) data shows that when all three markers for *Ry_{adg}* are in present in a clone, resistance is expressed against all PVY strains, including PVY^{N:O}, a newly documented strain in North America (Nie and Singh 2002; Singh et al. 2003). This supports previous reports in the literature of *Ry_{adg}* providing extreme resistance to PVY, defined as resistance against all strains (Munoz et al. 1975; Valkonen et al. 1994; Hamalainen et al. 1998). Use of the markers in the breeding clones/cultivars also shows a high correlation (98.1%) between marker presence and PVY resistant response in field and grafting evaluations (A85530-10 being the exception with a susceptible response to PVY^O grafting; Table 5). Sorri showed that the CAPS marker identified the *Ry_{adg}* gene in diverse backgrounds and at different ploidy levels, whereas another CAPS marker they tested detected resistant genotypes only within specific mapping population. This data is in agreement with Sorri by showing that when four diverse clones (Table 5; R241-16, A88597-7, A88625-10, A93575-4) with the ADG2/*BbvI* marker were graft inoculated with both O and N strains, they were immune and by phenotype represent extreme resistance. Six clones (Table 5; A88617-6, A95506-1, PA98V1-1, PA98V1-2, PA98V2-1, PA98V6-1) negative for the ADG2/*BbvI* marker were also graft-immune to O

and N strains, but their background includes *S. stoloniferum* not *S. tuberosum* ssp. *andigena*; four of the clones have only *S. stoloniferum* and the other two clones have *S. tuberosum* ssp. *andigena*, *S. stoloniferum*, and other species in their background.

Kasai et al. (2000) suggested that the SCAR markers (RYSC3 and RYSC4) might also be useful in detecting PVA as well as PVY resistance, because of the tight linkage (6.8 cM) to the *Ry_{adg}* gene (Hamalainen et al. 1998; Kasai et al. 2000). However, in the ten clones in this study with PVA resistance, only two of them were positive for the SCAR markers and had *S. tuberosum* ssp. *andigena* in their background. The remainder were negative for these markers with PVA resistance likely being conferred from *S. stoloniferum* (Table 5) (Barker 1996; Solomon-Blackburn and Barker 2001a).

The positive CAPS marker results for *S. pinnatisectum* indicate that there may be a homologous region with *S. tuberosum* ssp. *andigena*. Fifteen *S. pinnatisectum* accessions are listed in the National Germplasm Resources Laboratory database (USDA, ARS 2008). Of these fifteen, five have been tested for PVY resistance with two accessions resistant, one hypersensitive, and two susceptible. The hypersensitive response listed is for accession PI253214 while PVY resistance in *S. pinnatisectum* PI 275233 has not yet been determined (both are positive for the ADG2/*BbvI* marker in this study). The positive marker result in these two accessions is interesting in that Sorri et al. (1999) had previously reported its presence only in individuals expressing ER to PVY conferred by the presence of *Ry_{adg}*. Further evaluation of both accessions response is needed to determine reaction to multiple PVY

strains and if they follow an extreme or hypersensitive resistance pattern.

Kasai et al. (2000) points out that work is underway to provide breeders with MAS kits for resistant traits. Markers for PVY resistance have been developed and their use is being incorporated into breeding programs using *S. stoloniferum* markers (Milbourne et al. 1998; Song et al. 2005; Valkonen et al. 2008; Song and Schwarzfischer 2008) and *S. tuberosum* ssp. *andigena* markers (Sorri et al. 1999; Kasai et al. 2000; Dalla Rizza et al. 2006). However, traditional screening methods are still needed in the identification of PVY resistant germplasm with resistance conferred by genes divergent from *Ry_{adg}* and *Ry_{sto}*—useful for the successful pyramiding of genes conferring PVY resistance. An illustration of this is found in the case of Premier Russet, a potato cultivar reported as very resistant to PVY^O and having a reduced rate of infection to PVY necrotic strains (Novy et al. 2008). Use of RYSC3 and ADG2/*Bbv1* markers for *Ry_{adg}* and STM0003 (SSR) marker for *Ry_{sto}* showed no presence of R-genes from these species in Premier Russet (M.I. Vales, pers. com.).

In conclusion, the molecular markers linked to *Ry_{adg}* proved informative in identifying resistant individuals within a population segregating for extreme resistance to PVY. This finding supports the use of the three markers for MAS in a potato breeding program to accelerate the development of PVY resistant potato cultivars. In addition, a survey of diverse breeding clones and cultivars using the molecular markers was useful in determining whether observed PVY resistance was conferred by *Ry_{adg}* or by other resistance genes—useful information to potato breeders in the development of durable resistance to PVY by the pyramiding of divergent resistance genes.

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